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VEGFR1 and VEGFR2 in Alzheimer's disease

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Running title: VEGFR1 and VEGFR2 in Alzheimer's disease

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ABSTRACT

Vascular endothelial growth factor (VEGF) is a potent angiogenic factor. Despite upregulation of VEGF in the brain in Alzheimer's disease (AD), probably in response to A β , vasoconstriction and tissue hypoxia, there is no consequent increase in microvessel density. VEGF binds to and activates VEGF receptor 2 (VEGFR2), but also binds to VEGF receptor 1 (VEGFR1), which exists in less-active membrane-bound and inactive soluble (sVEGFR1) forms and inhibits pro-angiogenic signaling. We have investigated whether altered expression of VEGF receptors might account for the lack of angiogenic response to VEGF in AD. We assessed the cellular distribution and protein level of VEGFR1 and VEGFR2 in parietal cortex from 50 AD and 36 age-matched control brains, and related the findings to measurements of VEGF and von Willebrand factor (vWF) level (a marker of microvessel density) in the same tissue samples. VEGFR2 was expressed by neurons, astrocytes and endothelial cells. VEGFR1 was expressed predominantly neuronally and was significantly reduced in AD ($p = 0.02$). Western blot analysis on a subset of brains showed reduction in VEGFR1:sVEGFR1 in AD ($p = 0.046$). The lack of angiogenesis despite cerebral hypoperfusion in AD is not explained by altered expression of VEGFR2 or total VEGFR1; indeed, the down-regulation of VEGFR1 may represent a pro-angiogenic response to the hypoperfusion. However, the relative increase in sVEGFR1 would be expected to have an anti-angiogenic effect which may be a factor in AD.

Keywords: Alzheimer's disease, vascular endothelial growth factor A, vascular endothelial growth factor receptor-1, vascular endothelial growth factor receptor-2, brain ischemia, microvessels

INTRODUCTION

Blood flow and oxygenation are reduced in the cerebral cortex and underlying white matter in Alzheimer's disease (AD) [1-7]. Cerebral blood flow (CBF) declines before cognitive decline or brain atrophy [1, 8] and is associated with biochemical and MRI evidence of tissue damage [4, 9]. Vessel wall abnormalities such as cerebral amyloid angiopathy (CAA) and arteriolosclerosis, may play a part in the hypoperfusion but its timing and distribution suggest that other factors are more important contributors. These include cholinergic denervation [10, 11], reduced production of nitric oxide [12-15], and an increase in the intracerebral production of vasoconstrictors such as endothelin I and angiotensin II [16-19].

VEGF-A (VEGF) is a pro-angiogenic factor [20, 21] that is also involved in neuroprotection, neurogenesis, synaptic plasticity, and modulation of inflammation and astrocyte proliferation [22-26]. It is upregulated in response to hypoxia [27, 28] and elevated within hypoperfused cortex and white matter in small vessel disease, vascular dementia and AD [3, 29-32]. Its effects are mediated by the tyrosine kinase receptors, vascular endothelial growth factor receptors 1 and 2 (VEGFR1 and VEGFR2), neuropilin (NRP1 and NRP2) and heparan sulfate proteoglycans (HSPGs) co-receptors. The interactions in this complex receptor system were reviewed by Simons et al [33]. A β has been implicated in the direct upregulation of VEGF [34, 35] but this has been disputed [36].

The present study focuses on VEGFR1 and VEGFR2 in AD. VEGFR1 transduces signals for cellular proliferation and permeability, and is important in postnatal angiogenesis [37], neuroprotection [38, 39], neurogenesis [40] and microglial migration [41, 42]. VEGFR1 has a high affinity for VEGF, although its kinase activity is only around one tenth of that of VEGFR2 [43]. The VEGFR1 gene (FMS-like tyrosine kinase 1, *FLT1*) gives rise to multiple transcript variants that encode protein isoforms with different cellular localizations and properties. VEGFR1 splice isoforms can be membrane bound, soluble (sVEGFR1) [44] and

intracellular [45]. sVEGFR1 isoforms have no kinase domain and have been reported as being present in at least a 10-fold molar excess over VEGF, effectively suppressing VEGF-induced endothelial cell growth under normal conditions [43]. This suggests that VEGFR1 is an important negative regulator of VEGF activity [43, 46].

VEGFR2 is the main signal transducer for VEGF. The binding of VEGF results in receptor dimerization and autophosphorylation of the highly active receptor kinase region [47]. VEGFR2 was first identified as a major regulator of angiogenesis [48] and is now known to mediate endothelial cell proliferation, migration, permeability and survival [49-51]. In the adult nervous system it mediates neurogenesis [26], long term potentiation (LTP) [25], neuronal survival [23], astrocyte migration [52] and proliferation [53, 54].

The reported increase in VEGF in AD is not accompanied by a corresponding increase in microvessel density [3]. There are several possible explanations for this disparity. VEGF is partly sequestered in A β plaques in the AD cortex, where it binds to A β with a higher affinity than to its own receptors [55], probably reducing the bioavailability of VEGF. In addition, A β 42 was shown to be antiangiogenic *in vitro* and was subsequently found to bind to VEGFR2 and block receptor phosphorylation in human brain microvascular endothelial cells [56, 57].

Our aims in this study were to determine the cellular distribution and levels of VEGFR1 and VEGFR2 in human post-mortem cerebral cortex in AD and age-matched controls, and to investigate the relationship between receptor levels, VEGF level and microvessel density.

MATERIALS AND METHODS

Study cohort

Brain tissue was obtained from the South West Dementia Brain Bank (SWDBB), University of Bristol, UK with research ethics committee approval (NRES Committee South West - Central Bristol 08/H0106/28+5). All studies conformed to relevant regulatory standards. The brains had been divided mid-sagittally: the left hemisphere sliced as soon as

possible after removal, and then frozen at -80°C , and the right hemisphere fixed in formalin for three weeks before it was sampled for detailed neuropathological examination. Controls were defined by an absence of clinical history of cognitive decline or other neurological disease and a lack of neuropathological abnormalities apart from sparse neuritic or diffuse plaques in some cases. All controls were of Braak tangle stage III or lower. According to National Institute on Aging-Alzheimer's Association guidelines, AD neuropathological change was considered an adequate explanation for the dementia in all cases in the AD group [58]. CAA had been semi-quantitatively assessed in the parenchymal and meningeal vessels in five cerebral regions, including the parietal cortex. CAA severity was rated on the following scale: 0, none; 1, sparse or mild; 2, moderate; 3, frequent or severe [59]. Summary demographic and neuropathological data are shown in Table 1 and MRC UK Brain Banks Network database identifiers are listed in Supplementary Tables 1, 2 and 3. The dataset used in this study has been archived at <https://brainbanknetwork.cse.bris.ac.uk/studydata/ST0275> with links to all of the clinical and neuropathological information stored within the UK Brain Banks Network database on brains in the study cohort.

Tissue preparation

Approximately 200 mg samples of frozen parietal cortex (Brodmann area 40) were dissected and homogenized in 1% NP-40 buffer (1% NP-40, 20 mM Tris (pH 8.0), 137 mM NaCl, 10% glycerol, 2 mM EDTA, 1 mM activated sodium orthovanadate, 10 $\mu\text{g/mL}$ aprotinin, 10 $\mu\text{g/mL}$ leupeptin) in a Precellys 24 homogeniser (Stretton Scientific, Derbyshire, UK) with 2.3 mm ceramic beads (Biospec, Suffolk, UK) as previously described [3, 31]. The homogenates were spun at 13,000 g for 15 min at 4°C and the supernatant removed and stored at -80°C for subsequent assays. Total protein concentration was determined using Total Protein Kit (Sigma Aldrich, Dorset, UK) following the manufacturer's instructions. All assay measurements were adjusted for total protein level.

Antibodies

The primary antibodies were as follows: VEGFR1 (Ab32152, clone Y103, rabbit monoclonal, Abcam, Cambridge, UK); VEGFR2 (#2479, clone 55B11, rabbit monoclonal, Cell Signaling Technology, Danvers, MA, USA); glial fibrillary acidic protein (GFAP, M0761, clone 6F2, mouse monoclonal, Dako, Santa Clara, CA, USA); human leukocyte antigen-DP, DQ, DR (HLA-DR, M0775, clone CR3/43, mouse monoclonal, Dako).

Immunohistochemistry (IHC)

Seven-micrometer paraffin-embedded sections of brain tissue were dewaxed, hydrated and immersed in methanol, containing 3% hydrogen peroxide to block endogenous peroxidase activity. Sections were microwaved for 10 min in citrate buffer (10 mM trisodium citrate, 0.05% Tween 20 pH 6.0) for antigen retrieval before being blocked with 20% normal horse serum and incubated overnight with primary antibody optimally diluted in phosphate-buffered saline (PBS) (VEGFR1 100ng/ml, VEGFR2 260ng/ml). The following day, bound antibody was detected with biotinylated universal secondary antibody and visualized with avidin-biotin horseradish peroxidase (HRP) complex (ABC-kit, Vector Laboratories, Burlingame, CA, USA) and 3,3'-diaminobenzidine (DAB) containing 0.1% H₂O₂ (DAB, Vector Laboratories). Sections were immersed in 0.16 M copper sulfate to enhance staining. To detect a second antigen, sections were blocked with 20% normal horse serum, primary antibodies, (GFAP 230 µg/ml, HLA-DR 360 µg/ml) applied overnight at room temperature, and sections visualized with avidin-biotin HRP complex using VIP peroxidase substrate (Vector Laboratories). Sections were counterstained with Harris's hematoxylin. Immunoglobulin controls and negative controls with no primary antibody were tested alongside.

Validation of specificity of antibodies to VEGFR1

Specificity was assessed by western blot of brain homogenates. Membrane was blocked with 3% BSA for 1 hour and incubated overnight at 4°C with primary antibody.

VEGFR1 antibody was at a concentration of 10 ng/ml in 3% bovine serum albumin (BSA) (Sigma Aldrich) in Tris-buffered saline-Tween (TBS- 0.05% Tween 20); VEGFR2 antibody was used at a concentration of 26 ng/ml in 3% BSA in TBS-T. β -Actin was used as a loading control. Human umbilical vein endothelial cell (HUVEC) lysates were used as a positive control. Recombinant VEGFR1 and VEGFR2 (R&D) were used to confirm the specificity of the antibody.

Calculation of VEGFR1:sVEGFR1 ratio

Western blot was performed on 12 control and 12 AD samples selected at random from the cohort. Membranes were developed and analyzed using BioRad ImageLab software (Version 5.2, Bio-Rad Laboratories, Hercules, CA, USA), and lanes and bands were automatically detected, and then checked and the selection adjusted manually if required. Molecular weights of sample bands were calculated using protein ladder(s) as a reference. The volume of intensities within the band boundaries were used to calculate the ratio of membrane bound VEGFR1 to the sum of its soluble forms.

VEGFR1 dot blot

After the specificity of the VEGFR1 antibody had been confirmed by western blot on recombinant protein and human brain tissue homogenates, total VEGFR1 level across the whole cohort was determined by dot blot. The method was similar to that previously described [3, 31, 60]. Samples were diluted in TBS (1:1000) and blotted onto nitrocellulose membrane for 1 h at room temperature. The membrane was blocked in 5% BSA in TBS-T for 1 h at room temperature, washed, and then incubated overnight with VEGFR1 antibody diluted 10 ng/ml in 3% BSA TBS-T, at 4 °C with agitation. After washing, the membrane was incubated with anti-rabbit peroxidase-conjugated secondary antibody (Vector Laboratories) in 5% non-fat dried milk protein diluted in TBS-T for 1 h at room temperature, with agitation. The membrane was washed and then developed with chemiluminescent ECL substrate (Millipore, Billerica, MA, USA). BioRad Image Lab (Version 5.2, BioRad, Hercules, CA, USA)

was used to measure the integrated density of each sample. Serial dilutions of a standard reference brain tissue homogenate were added to each blot and used to adjust for any blot-to-blot variation.

VEGFR2 sandwich ELISA

VEGFR2 protein level was measured using Human Total VEGFR2 (KDR) DuoSet IC ELISA kit (DYC1780, R&D Systems) according to the manufacturer's guidelines. To validate the ELISA for use on human brain tissue, samples were spiked with 1000 pg/ml of VEGFR2 recombinant protein; percentage recovery and linearity were within the acceptable range of 80-120% as stated by the manufacturer. 50 µl samples were added in duplicate and incubated for 2 h at room temperature. Absorbance was measured at 450 nm in a FLUOstar Optima plate reader (BMG Labtech, Ayelsbury, UK) and VEGFR2 concentration was interpolated from a standard curve generated by serial dilution of human recombinant VEGFR2 (62.5–4000 pg/ml).

Von Willebrand factor sandwich ELISA

Von Willebrand factor (vWF) level was determined using vWF Human ELISA kit (ab108918, Abcam) according to the manufacturer's guidelines. The ELISA used a vWF capture antibody and a biotinylated vWF detection antibody. 50 µl samples were added in duplicate and incubated for 2 h at room temperature. Absorbance was measured at 450 nm and vWF concentration was interpolated from a standard curve generated by serial dilution of human recombinant vWF (2.5–80 mU/ml).

VEGF sandwich ELISA

VEGF protein level was measured using Human VEGF DuoSet ELISA kit (DY293B, R&D Systems) as previously described [3] and according to the manufacturer's guidelines. The ELISA used a VEGF capture antibody and a polyclonal biotinylated VEGF detection antibody. 50 µl samples were added in duplicate and incubated for 2 h at room temperature. Absorbance was measured at 450 nm and VEGF concentration was interpolated from a

standard curve generated by serial dilution of human recombinant VEGF (31.25–2000 pg/ml).

Statistical analysis

Mann Whitney tests were used for comparisons between groups (as the data were not normally distributed) and Spearman's test to assess rank order correlation. Spearman's test to was used to assess rank order correlation. The analyses were performed using GraphPad Prism (Version 6.00, GraphPad Software, La Jolla, CA, USA). *P*-values of < 0.05 were considered statistically significant.

RESULTS

VEGFR1 is predominantly neuronal

VEGFR1 was readily detectable immunohistochemically in the neuropil of the cerebral cortex in AD (Fig. 1A) and control brains, with most intense immunolabelling of neuronal cell bodies (Fig. 1B, C). There was minimal co-localization with GFAP-positive astrocytes (Fig. 1B) or HLA-DR-positive microglia (Fig. 1C). There was also minimal labelling of blood vessels (not shown). Antibody specificity to VEGFR1 in human tissue is shown in Supplementary Fig. 1.

VEGFR1 is decreased in Alzheimer's disease

We measured the level of VEGFR1 in the parietal cortex (Brodmann area 40) of AD and control brains. The median level of VEGFR1 was significantly lower in AD than control brains ($p = 0.02$; median in AD = 61.85, interquartile range = 43.19; median in controls = 76.39, interquartile range = 42.43) although there was substantial overlap between the two groups (Fig. 1D).

Reduction in VEGFR1:sVEGFR1 in AD cortex

Western blots with antibody to the N terminus of VEGFR1 were used to quantify membrane bound and soluble forms of VEGFR1 (Fig. 2A). VEGFR1 migrates at 180 kDa and sVEGFR1 isoforms range from 80-135 kDa. There were no prominent bands below 75 kDa, as illustrated in Supplementary Fig. 2. VEGFR1:sVEGFR1 was significantly lower in AD than controls, ($p = 0.046$; median ratio in AD = 0.23, interquartile range = 0.87; median ratio in controls = 3.39, interquartile range = 4.32) (Fig. 2B).

VEGFR2 is present mainly within astrocytes and endothelial cells

Double-labelling of VEGFR2 and either GFAP or HLA-DR in AD and control brains showed VEGFR2 to be present in astrocytes, endothelial cells and the tunica media of larger vessels (Fig. 3). In endothelial cells, antibody to VEGFR2 labelled the cytoplasmic membranes. There was also some immunolabelling of neuronal cell bodies but not the surrounding neuropil. VEGFR2 was not detected within HLA-DR-positive microglia. Antibody specificity to VEGFR2 in human tissue is shown in Supplementary Fig. 3.

VEGFR2 level is unchanged in AD

VEGFR2 was measured by ELISA. The levels did not differ significantly between control and AD homogenates ($p = 0.42$; median in AD = 394.9, interquartile range = 491.1; median in controls = 328.8, interquartile range = 314.1) (Fig. 3D).

Vascularity remained unchanged in AD and was not related to VEGF

To ensure that changes in receptor level were not due to a reduction in blood vessel density in AD, we measured vWF, a surrogate measure of blood vessel density, in the same parietal cortex homogenates. There was no difference in the level of vWF between AD and control brains ($p = 0.143$, Fig. 4A). As VEGFR1 is normally upregulated by hypoxia [61, 62], we also measured VEGF level in the homogenates, as an indicator of hypoxia. There was a trend towards an increase in VEGF in the AD brains, in keeping with previous observations [3] although this did not reach significance ($p = 0.069$; median in AD = 44.35, interquartile

range = 21.77; median in controls = 34.65, interquartile range = 39.00) (Fig. 4B). There was no correlation between VEGF and vWF in control ($p = 0.525$, $r = 0.119$, Fig. 4C) or AD brains ($p = 0.437$, $r = -0.119$). The receptor levels were correlated with to age and post-mortem delay to rule out

Correlations between VEGFR1 and VEGFR2 and age and postmortem delay were performed to account for their effect on changes in receptor levels and no correlation was found (Supplementary Fig. 4). The relationship between VEGFR level and CAA score was also examined and there was no difference between cases with little or sparse CAA and those with moderate to severe CAA (Supplementary Fig.5).

DISCUSSION

This study set out to establish whether altered expression of VEGFR1 and VEGFR2 might account for the lack of angiogenic response to cerebral hypoperfusion in AD. VEGFR1 was shown to be expressed mainly by neurons, and VEGFR2 by neurons, endothelial cells and astrocytes. We found the level of VEGFR2 to be unchanged in the parietal cortex in AD. Unexpectedly, the total level of VEGFR1, thought to be a negative regulator of VEGF activity, was reduced in AD, although the cortex contained proportionally more of the lower-molecular weight, soluble, inactive isoforms of the receptor.

The expression of VEGFR1 and VEGFR2 by neurons is in keeping with previous evidence of the involvement of VEGF in neurogenesis, neuronal migration and synaptic plasticity [22-26, 40]. VEGFR2 was previously identified in astrocytes under hypoxic conditions [63, 64], and in a subset of astrocytes in AD [29] and in human post-mortem brain tissue from patients with small vessel disease and controls [65]. VEGFR1 was reported to play a role in microglial chemotaxis and proliferation [42, 66]. We did not detect convincing VEGFR1 immunopositivity within microglia in either AD or control brains, in contrast to the findings of Ryu et al [2009]. The reason for the disparity is not clear but could include their

use of a different VEGFR1 antibody whose specificity was not confirmed, and differing fixation protocols [41].

VEGFR1, like VEGF, is upregulated by hypoxia [61, 62, 67] and our expectation was therefore that VEGFR1 production would increase in AD. However, contrary to this, we found a reduction in total VEGFR1 level in AD, and this may reflect post-translational modifications and increased protein degradation. Ikeda et al [2011] previously demonstrated downregulation of VEGFR1 in human microvascular endothelial cells under conditions of hypoxia [68]. The reduction in total VEGFR1 was attributed to a reduction in sVEGFR1 mRNA and protein, while the level of membrane bound VEGFR1 remained unchanged [68]. If oxygenation is chronically reduced, the downregulation of sVEGFR1 may be physiologically beneficial as, through sequestration of VEGF, these inactive shorter forms of VEGFR1 are thought to be strong negative regulators of angiogenesis. In this study, membrane-bound VEGFR1 was reduced in AD brain, in agreement with dot blot results. When we examined the ratio of membrane bound VEGFR1 to sVEGFR1 we found a decrease in AD compared to controls and indicates an increase in the proportion of sVEGFR1 isoforms.

We found VEGFR2 level to be unchanged in AD compared to control brains. Studies of VEGFR2 expression in animal models of brain ischemia and *in vitro* models of neuronal or brain tissue hypoxia have yielded conflicting results. In some, VEGFR2 was upregulated [69-73] and in others, decreased [74-76]. Other factors that may account for the reduced or unchanged microvessel density in hyperperfused cortex in AD [3] include sequestration of VEGF by A β 42 [55], the decreased VEGFR1:sVEGFR1 ratio (see above), and binding of A β 42 to VEGFR2 – shown by Patel et al [2010] to block VEGF-mediated signaling. There was no difference in vWF level between control and AD brain homogenates, indicating that the reduction in VEGFR1 level and maintenance of VEGFR2 level was not due to a change in vessel density. VEGF was previously shown to be increased in the frontal cortex in AD compared to control brains, in keeping with other evidence of reduced tissue

oxygenation [3]. In this study, we saw a trend towards an increase in VEGF in the parietal cortex in the AD cases and no correlation between VEGF and VWF in AD.

Our interpretation of the relevance of VEGFR1 and VEGFR2 in the context of vascular homeostasis and hypoperfusion of the parietal cortex in AD is summarized in Fig. 5. However, there are likely to be wider implications of disruption of the VEGF-VEGFR signaling pathway in AD. In particular, VEGF is essential for long-term potentiation (LTP) and increases synaptic strength independent of its effects on angiogenesis and neurogenesis [25]. Interference with VEGF signaling may contribute to the inhibitory effects of A β 42 on synaptic transmission [77-79]. Indeed, VEGF administration has been investigated as a neuroprotective, proangiogenic therapy in AD [80-82]. The present study adds to our understanding of VEGF-VEGFR signaling in AD. However, our analysis is based on a cohort of modest size, and the findings require confirmation. In addition, further work is needed to determine precisely why this signaling pathway does not function normally.

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CONFLICT OF INTEREST

The authors have no conflict of interest to report.

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Table 1. Summary demographic data of the control and AD cases used in the study

Parietal cortex		C	AD
N		36	50
Age	Range	58-95	57-93
	Mean \pm standard deviation	80.1 \pm 8.7	77.5 \pm 8.1
Gender	Female	11	26
	Male	25	24
Post-mortem delay (hours)	Range	3 - 67	4 - 85
	Mean \pm standard deviation	32.5 \pm 16.3	31.6 \pm 19.0

Figures

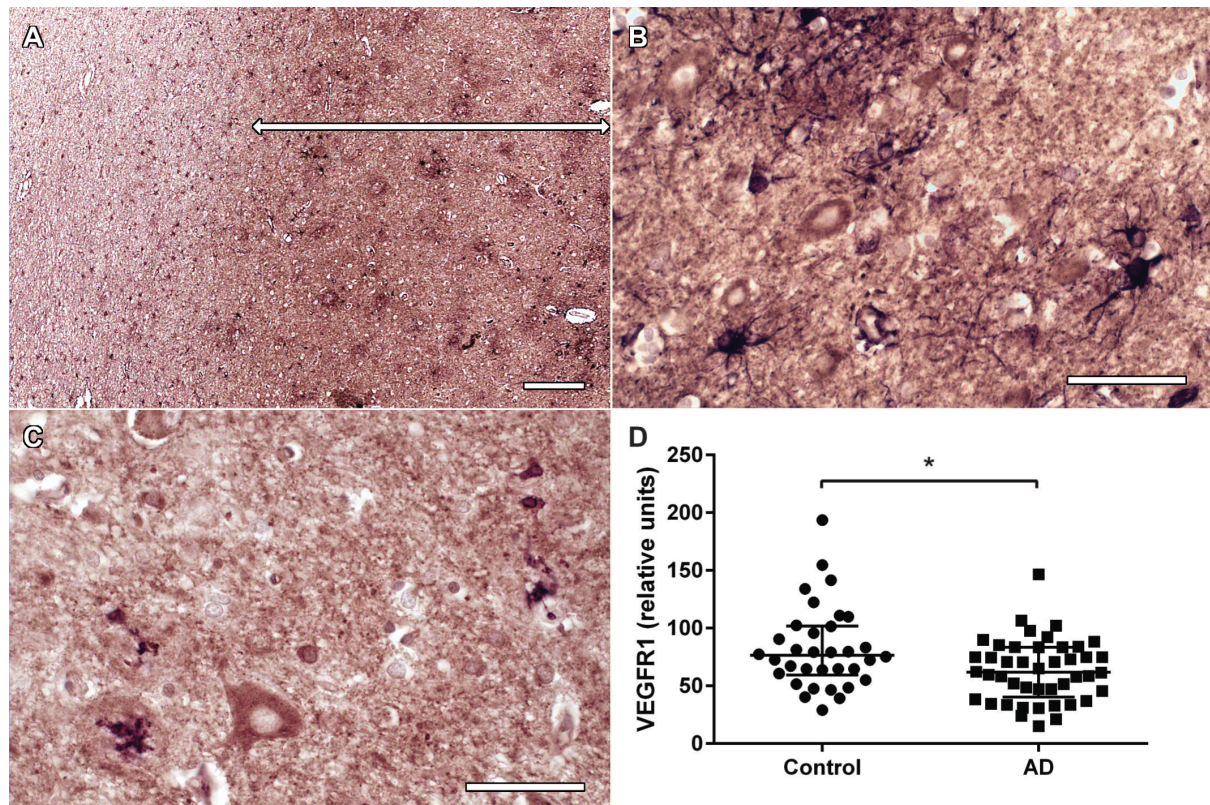


Fig. 1. Cellular localization and level of VEGFR1 in human parietal cortex. The examples shown are from an AD brain. (A) Double-labelling of VEGFR1 (brown) and GFAP (purple) shows VEGFR1 to be present in the cerebral cortex (double-headed arrow) but not the underlying white matter. Bar = 2 mm. (B) At higher magnification VEGFR1 is detectable within neurons and neuropil, with minimal labelling of GFAP-positive astrocytes. Bar = 50 μm. (C) Double-labelling of VEGFR1 (brown) and HLA-DR (purple) shows minimal VEGFR1 immunopositivity in HLA-DR-positive microglia. Bar = 50 μm. (D) VEGFR1 protein level (measured by dot blot) was significantly lower in AD than control brains ($p = 0.02$). Each point represents a single brain. The horizontal lines indicate the median \pm interquartile range.

* $p < 0.05$.

Fig. 2. VEGFR1 western blots on control and AD parietal cortex homogenates. (A) Membrane bound VEGFR1 was observed at 180 kDa. sVEGFR1 isoforms were observed between 80-135 kDa. β -actin was used as a loading control. (B) Scatterplots showing VEGFR1:sVEGFR1 in control and AD parietal cortex, as determined from integrated area-density measurement of the western blot bands. The horizontal lines indicate the median \pm interquartile range. The ratio was significantly lower in the AD brains. * $p < 0.05$.

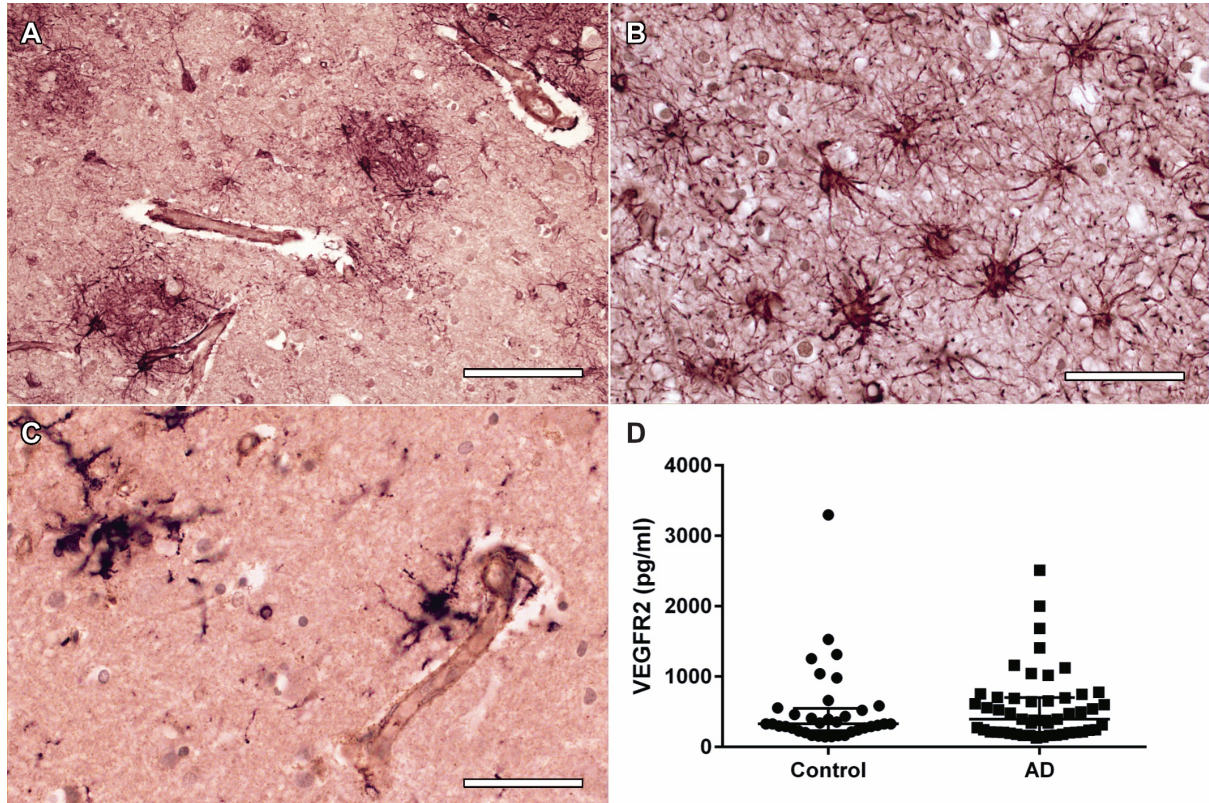


Fig. 3. Cellular localization and level of VEGFR2 in an AD brain. (A) Double-labelling of VEGFR2 (brown) and GFAP (purple) reveals VEGFR2 within endothelial cells and the tunica media of larger vessels, and also in neurons and GFAP-positive astrocytes. Bar = 100 μ m. (B) This image shows more clearly the combination of brown (VEGFR2) and purple (GFAP) reaction product within astrocytes. Bar = 50 μ m. (C) Double-labelling of VEGFR2 (brown) and HLA-DR (purple) does not show convincing co-localization of these antigens within microglia. A capillary shows a membranous pattern of VEGFR2 immunolabelling. Bar = 50 μ m. (D) VEGFR2 protein level (measured by ELISA) did not differ significantly between control and AD homogenates ($p = 0.42$). Each point represents a single brain. Median \pm interquartile range are also displayed.

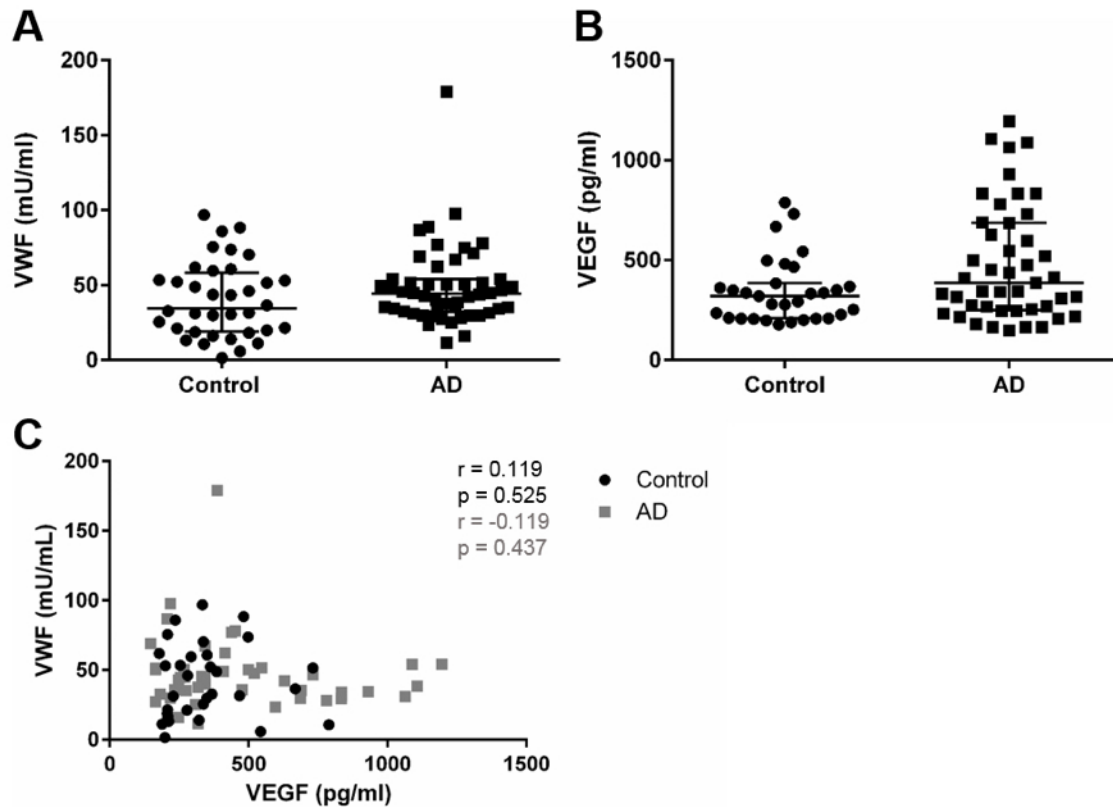


Fig. 4. VEGF in relation to vWF, VEGFR1 and VEGFR2. (A) Scatterplots showing vWF level in the homogenates of parietal cortex. The horizontal lines indicate the median \pm interquartile values. The level of vWF did not differ significantly between control and AD brains (Mann-Whitney test, $p = 0.143$). C: $n = 36$, AD: $n = 50$. (B) VEGF level was higher in AD than control brains although this did not reach significance (Mann-Whitney test, $p = 0.069$). C: $n = 31$, AD: $n = 45$. (C) No correlation between VEGF and vWF in control ($p = 0.525$, $r = 0.119$, $n = 31$) or AD ($p = 0.437$, $r = -0.119$, $n = 45$) parietal cortex. Each point represents one brain.

* $p < 0.05$.

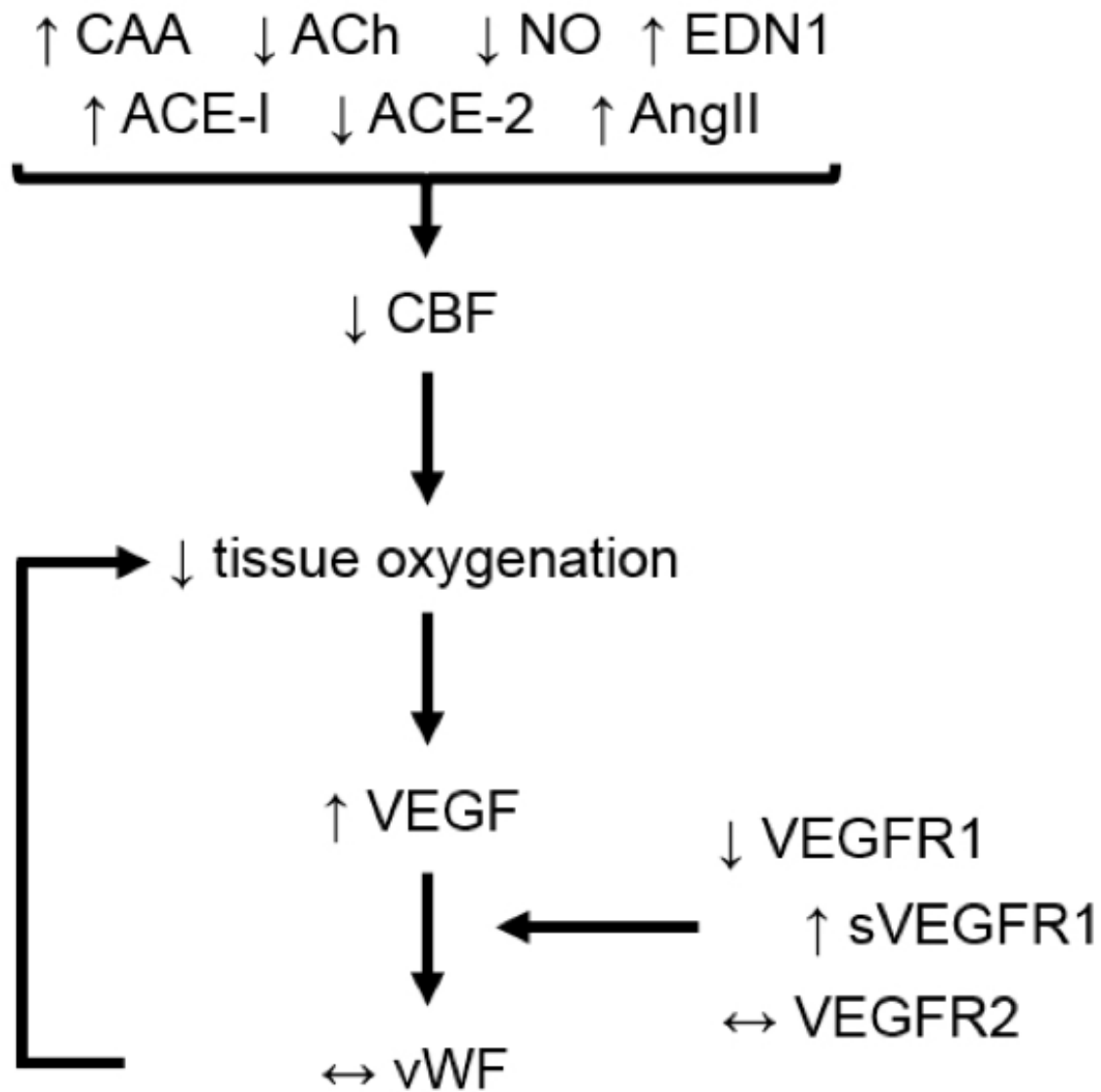


Fig. 5. Hypothesized relevance of present findings in the context of vascular homeostasis and hypoperfusion of the parietal cortex in AD. Cerebral amyloid angiopathy (CAA), decreased cholinergic innervation of intracerebral bloodvessels [10, 11], reduced NO production [12-15] and an increase in vasoconstrictors EDN1 [16, 19] and AngII, through upregulation of angiotensin-converting enzyme (ACE)-1 and downregulation of ACE-2 [17, 18]) may all contribute to reduced cerebral blood flow (CBF). Reduced tissue oxygenation leads to increased VEGF [3, 29-32] that is not accompanied by an increase in endothelial von Willebrand factor (vWF) level and microvessel density [3]. Reduction in VEGFR1 is probably a physiological response to reduced oxygenation but an increase in the soluble

form of the receptor protein (sVEGFR1) may contribute to impaired angiogenesis. VEGFR2 level remains unchanged. Arrows denote an increase (\uparrow) or decrease (\downarrow) in protein in AD compared to control. Horizontal double arrow (\leftrightarrow) indicates no change in AD compared to controls.